(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 October 2002 (17.10.2002)

PCT

(10) International Publication Number
WO 02/081660 A1

(51) International Patent Classification[7]: C12N 5/00, C07K 14/47

(21) International Application Number: PCT/CA02/00486

(22) International Filing Date: 3 April 2002 (03.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/280,850 3 April 2001 (03.04.2001) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY HEALTH NETWORK** [CA/CA]; 610 University Avenue, Toronto, Ontario M5G 2M9 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ERWIN, W., Mark** [CA/CA]; 23 Lochinvar Cres., Toronto, Ontario M3A 3J9 (CA). **SALO, Paul, T.** [CA/CA]; 3819-18th Street Southwest, Calgary, Alberta T2T 4V2 (CA). **INMAN, Robert** [CA/CA]; 118 Hillsdale Ave. W., Toronto, Ontario M5P 1G5 (CA).

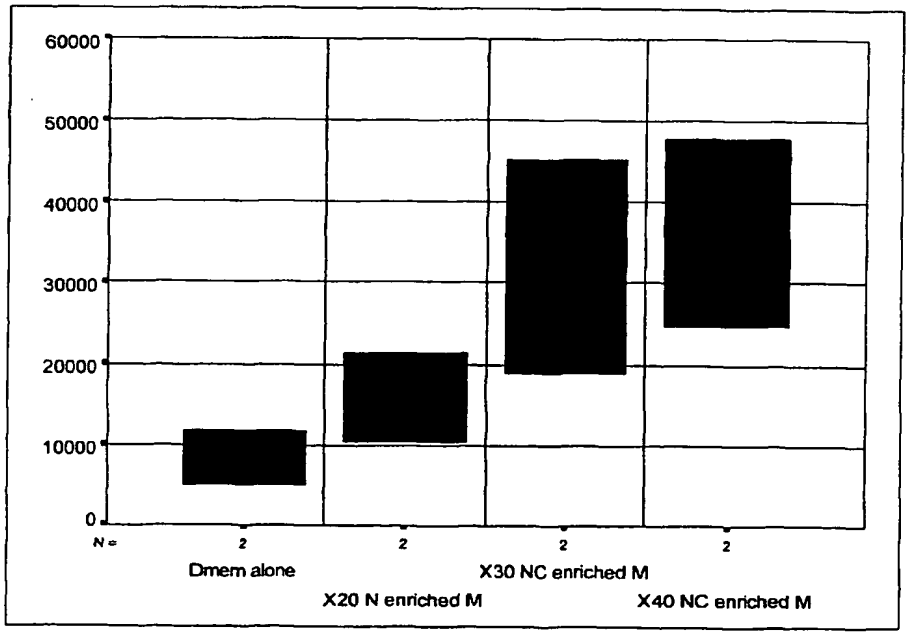
(74) Agent: **BERESKIN & PARR**; 40 King Street West, Box 401, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent

[Continued on next page]

(54) Title: COMPOSITIONS AND METHOD FOR ENHANCING PROTEOGLYCAN PRODUCTION



(57) Abstract: The present invention provides compositions comprising notochord enriched media and/or one or more factors derived therefrom. Such compositions are useful for enhancing the production of proteoglycan in cells or animals in need thereof, for example for treating degenerative disc disease. The notochord enriched media is preferably obtained from a nonchondrodystrophic animal.

WO 02/081660 A1

(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *with international search report*

TITLE: COMPOSITIONS AND METHOD FOR ENHANCING PROTEOGLYCAN PRODUCTION

FIELD OF THE INVENTION

The invention relates to compositions and methods for enhancing proteoglycan production. Specifically, the present invention provides a composition comprising notochord enriched media and/or factors derived from notochord enriched media, and the use of such compositions to enhance proteoglycan production in chondrocytic cells.

BACKGROUND OF THE INVENTION

Degenerative disc disease is one of the most common causes of disability in North American society. The intervertebral disc is an avascular structure made of a sparse amount of cells interspersed in an extracellular matrix composed of mainly collagen, proteoglycan and water. During the aging process the disc experiences certain biochemical, structural and morphological changes. The effects of these changes are most significant in the nucleus pulposus, which is where many believe that disc degeneration begins. Some of the factors implicated in these changes are disc cell nutrition, degradative enzymes, inflammatory mediators, apoptosis and prolonged mechanical loading. A decrease in cell viability and changes in the matrix composition of the intervertebral disc are visible signs of degenerative disc disease that may be detected during the aging process.

Not every person develops degenerative disc disease. There is no biological explanation for the disparity in people who do and do not develop degenerative disc disease in the absence of trauma. An important observation in this regard is that some animals do not develop degenerative disc disease and it is these species that maintain a population of disc notochord cells into adult life. The canine species is a case in point with respect to factors that may have a genetic link. Nonchondrodystrophic dogs maintain their notochord cells for many years and are not known to develop degenerative disc disease, whereas other species of purebred dogs such as beagles (the chondrodystrophic breeds) do develop degenerative disc disease.

It is considered that the loss of aggregating proteoglycan and the loss of the associated water content of the nucleus leads to a loss of the resiliency of the disc and compromised load-bearing capacity. The result of such matrix degeneration is further internal derangement of the nucleus, which seems to parallel the molecular disorganization of the nucleus extracellular matrix. It has been reported that the non-aggregating proteoglycans, that seem to arise in the process of degenerative disease, lack a binding site at the hyaluronan central protein core. Therefore, the development of substances that can enhance the production of aggregating proteoglycan can lead to effective treatments for degenerative disc disease and other disorders that involve degeneration of the matrix of chondrocytic cells.

SUMMARY OF THE INVENTION

The inventors have found that notochord enriched media and/or factors derived therefrom, stimulate proteoglycan production in bovine disc chondrocytes. As well, the inventors have verified the expression of several genes important to chondrocyte metabolism that are increased in expression as a result of culture with these factors. These genes have been shown to be active in bovine and human disc chondrocytes. Therefore, the present invention relates to a composition for enhancing the production of proteoglycan in a cell or animal in need thereof comprising notochord enriched media and/or one or more factors derived from notochord enriched media. Preferably the notochord enriched media, and/or one or more factors derived therefrom, are from a nonchondrodystrophic animal, for example, nonchondrodystrophic canines, rabbits or felines, and the cell is a chondrocytic cell, such as a chondrocyte from the disc or articula cartilage.

The present invention further involves a method for enhancing proteoglycan production comprising administering to a cell or animal in need of such treatment, an effective amount of a composition comprising notochord enriched media and/or one or more factors derived therefrom. The invention also relates to a use of a composition comprising notochord enriched media and/or one or more factors derived therefrom to enhance the production of proteoglycan in a cell or animal in need thereof, and a use of a composition

comprising notochord enriched media and/or one or more factors derived therefrom to prepare a medicament to enhance the production of proteoglycan in a cell or animal in need thereof. Preferably the cell is a chondrocyte and the subject is a mammal, in particular, humans.

In an embodiment of the present invention, the cell is an intervertebral chondrocyte, therefore there is provided a method of treating degenerative disc disease comprising administering to a cell or animal in need of such treatment, an effective amount of a composition comprising notochord enriched media and/or one or more factors derived therefrom. The invention also relates to a use of a composition comprising notochord enriched media and/or one or more factors derived therefrom to treat degenerative disc disease in a cell or animal in need thereof, and a use of a composition comprising notochord enriched media and/or one or more factors derived therefrom to prepare a medicament to treat degenerative disc disease in a cell or animal in need thereof.

The present invention further relates to a pharmaceutical composition for enhancing the production of proteoglycan comprising notochord enriched media and/or one or more factors derived therefrom and a pharmaceutically acceptable carrier.

The present invention further relates to a method of preparing notochord enriched media comprising:

(a) separating a nucleus pulposus from an intervertabal disc of a nonchondrodystrophic animal to provide a total nucleus digest;

(b) separating notochord cells from the total nucleus digest; and

(c) purifying the notochord cells and culturing the notochord cells in a media to provide notochord enriched media.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and

scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 is a plot of proteoglycan production as a function of concentration of NCEM applied to bovine disc chondrocytes (Y-axis is counts per minute, X-axis reflects concentration of enriched media).

Figure 2 is a plot of cell proliferation as a function of concentration of NCEM applied to disc chondrocytes as compared to DMEM only and DMEM with 10% fetal calf serum.

Figure 3 is a Tris-glycine SDS-PAGE of notochord enriched media, stained with colloidal Coomassie blue.

Figure 4 is a 1.7% agarose gel of PCR products from bovine disc chondrocytes cultured with NCEM; primers directed against aggrecan, versican, CD-44 and hyaluronan synthase.

Figure 5 is a 1.7% agarose gel of PCR products from human disc cultured with NCEM; primers directed against aggrecan, versican, CD-44 and link protein

DETAILED DESCRIPTION OF THE INVENTION

(i) Compositions

Maintenance of intervertebral disc integrity is dependent upon the interaction of the resident cell population, notochord and chondrocyte cells, and factors produced by notochord cells play a critical role in disc structure and function by exerting an anabolic effect on intervertebral disc chondrocytes. The inventors have partially characterized the soluble anabolic factors found in canine notochord enriched media and have found that these factors, in a dose dependent relationship, up-regulate the production of disc chondrocyte proteoglycans, specifically the large, presumably aggregating species. The present inventors have also shown that several genes important to chondrocyte metabolism are increased in expression in both human and bovine disc chondrocytes that have been cultured with notochord enriched

media. These proteoglycans provide the disc with an inherent ability to maintain its matrix and therefore avoid the internal disruption which otherwise proceeds inexorably with age and/or trauma.

The present invention therefore provides compositions for enhancing the production of proteoglycan in a cell or animal in need thereof comprising notochord enriched media and/or one or more factors derived from notochord enriched media.

Enhanced production of proteoglycan provides a chondrocytic cell with an ability to maintain its matrix. Accordingly, in an embodiment of the present invention, there is provided a composition for the treatment of degenerative disease of the chondrocyte matrix in a cell or animal in need thereof, comprising notochord enriched media and/or one or more factors derived from notochord enriched media. The chondrocytic cell may be any such cell, including, but not limited to intervertebral chondrocytes and chondrocytes from the articulate cartilage.

In embodiments of the present invention the chondrocyte is an intervertebral cell, the degeneration of which is a factor in degenerative disc disease. The present invention therefore provides compositions for the treatment of degenerative disc disease comprising notochord enriched media and/or one or more factors derived therefrom.

By "enhancing the production of proteoglycan" it is meant to increase, enhance or stimulate the amount of proteoglycan in the cell or animal when compared to a control. As used herein, the term "control" refers to a cell or animal under same conditions except a composition comprising notochord enriched media, and/or one or more factors derived from notochord enriched media, has not been administered thereto.

As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease

progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

"Palliating" a disease or disorder means that the extent and/or undesirable clinical manifestations of a disorder or a disease state are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disorder.

The term "notochord enriched media" as used herein refers to media enriched in factors isolated from a notochord cell culture system. Preferably, the notochord enriched media is derived from the notochord cells of a nonchondrodystrophic animal. A nonchondrodystrophic animal is an animal which does not develop degenerative disc disease and maintains a population of disc notochord cells into adult life. Such animals include, but are not limited to, canine, rabbit or feline nonchondrodystrophic species. Preferably such animals include canine nonchondrodystrophic species.

The factors derived from notochord enriched media may be any substance which modulates the expression of proteins involved in the synthesis and/or assembly of proteoglycan in chondrocytes. Such substances may include small molecules, DNA, RNA, lipids, proteins and peptides. Preferably the factors derived from notochord enriched media are soluble anabolic proteins or peptides. Specific soluble anabolic peptides from the notochord enriched media, suitable for the compositions of the present invention, are mainly in the 25-220 kilodalton size and occupy the neutral to acidic pH range. A list of the peptides produced by notochord cells *in vitro*, as sequenced by mass spectroscopy, that are suspected to be involved in whole or in part with the biologic activity of NCEM is found in Table 1. Some of the main proteins identified include alpha-2-HS-glycoprotein (Fetuin), TGF-beta-receptor-lie protein and alpha fetal proteins.

The compositions of the invention are administered to cells or animals in a biologically compatible form suitable for pharmaceutical administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is

meant a form of the notochord enriched media, or factors derived therefrom, to be administered in which any toxic effects are outweighed by the therapeutic effects of the media, or factors derived therefrom. The term animal is intended to include living organisms in need of treatment for degenerative disc disease, e.g., mammals. Examples of animals include humans, canine and equine species.

The compositions comprising notochord enriched media, or factors derived therefrom, may be administered in a convenient manner such as by injection (percutaneous, subcutaneous, intravenous, etc.), oral administration inhalation, transdermal application or rectal administration. Depending on the route of administration, the active compounds may be coated in a material to protect the compounds from the action of enzymes, acids and other natural conditions which may inactive the compounds. Preferably the compositions of the invention are administered by percutaneous injection.

The compositions of the invention to be administered to a subject may further comprise an appropriate carrier, or may be co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Accordingly, the present invention further relates to a pharmaceutical composition for enhancing the production of proteoglycan comprising notochord enriched media and/or one or more factors derived therefrom and a pharmaceutically acceptable carrier.

The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. Suitable carriers are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

To administer the active ingredients of the composition of the invention by other than parenteral administration, it may be necessary to coat the composition with, or co-administer the composition with, a material to prevent its inactivation. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in oil-in-water emulsions as well as conventional liposomes (Strejan et al.,

(1984) J. Neuroimmunol 7:27). The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The pharmaceutically acceptable carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, asorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating active compounds (e.g., notochord enriched media or factors derived therefrom) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compounds into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of

sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When the active compounds (e.g., notochord enriched media or factors derived therefrom) are suitably protected, as described above, the composition may be orally administered, for example, with an inert diluent or an assimilable edible carrier. Pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound(s), use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compounds (e.g., notochord enriched media or factors derived therefrom) calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compounds and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such active compounds for the therapeutic treatment of individuals. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (1990 - 18th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

The invention further contemplates compositions for enhancing the production of proteoglycan in a cell or animal in need thereof comprising one or more factors derived from notochord enriched media, for example, one or more of the peptides/proteins in Table 1. Possible proteins identified that may be useful in a composition for enhancing the production of proteoglycan in a cell or animal in need thereof include alpha-2-HS-glycoprotein (Fetuin), TGF-beta-receptor-lie protein and alpha fetal proteins. When the one or more factor derived from notochord enriched media is a protein or peptide, the protein or peptide may be modified to be more therapeutically effective or suitable. For example, the protein or peptide may be converted into pharmaceutical acceptable salts by reacting with inorganic acids including hydrochloric acid, sulphuric acid, hydrobromic acid, phosphoric acid, etc., or organic acids including formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid, benzenesulphonic acid, and tolunesulphonic acids. The protein or peptide may also be converted into a solvate. The term "solvate" as used herein means a protein or peptide, or a pharmaceutically acceptable salt of a protein or peptide, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate". The protein or peptide may also be converted into prodrugs.

The protein or peptide may be prepared using standard peptide synthesis chemistry (for example as described in "The Chemical Synthesis of Peptides" John Jones, Clarenden Press, 1991) or using recombinant DNA technology (for example as set out in Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989; and "Current Protocols in Molecular biology, Eds. Ausubel, FM. *et al.* (1994) John Wiley & Son). The protein or peptide may also be isolated from the notochord enriched media.

The formation of a desired peptide or protein salt is achieved using standard techniques. For example, the neutral peptide or protein is treated with an acid in a suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

The formation of solvates of the protein or peptide will vary depending on the peptide or protein and the solvate. In general, solvates are formed by dissolving the protein or peptide in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

Prodrugs of the protein or peptide may be conventional esters formed with available hydroxy, thiol, amino or carboxyl groups. For example, an available hydroxy, thiol, or amino may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g. an acid chloride in pyridine). Also, an available "C(O)OH" group in a peptide or protein of the invention, an ester may be formed by activation of the hydroxyl group of the acid and treatment with the appropriate alcohol in the presence of a base in an inert solvent. Some common esters which have been utilized as prodrugs are phenyl esters, aliphatic (C_8-C_{24}) esters, acyloxymethyl esters, carbamates and amino acid esters.

(ii) Method of Preparing the Notochord Enriched Media

As stated above, notochord enriched media as used herein refers to media enriched in factors isolated from a notochord cell culture system. Broadly stated, the present invention therefore provides a method of preparing notochord enriched media comprising:

(a) providing isolated notochord cells; and

(b) culturing the notochord cells in a medium suitable for maintaining the notochord cells.

The notochord cells are preferably from a nonchondrodystrophic animal. The notochord cells may be obtained, for example, from the nucleus pulposus which is found in the intervertabral disc. The medium suitable for maintaining the notochord cells may be, for example, Dulbecco's Modified Eagle Medium (DMEM).

In an embodiment of the present invention there is provided a method of preparing notochord enriched media comprising:

(a) separating a nucleus pulposus from an intervertabral disc of a nonchondrodystrophic animal to provide a total nucleus digest;

(b) separating notochord cells from the total nucleus digest; and

(c) purifying the notochord cells and culturing the notochord cells in media to provide notochord enriched media.

In an embodiment of the present invention, the notochord enriched media is prepared as described in Example 1 herein. In this embodiment, the notochord cells are separated from the nucleus pulposus from an intervertabral disc of a nonchondrodystrophic animal using a Percoll gradient method, for example as described in Example 1. Once separated, the notochord cells must be separated from the Percoll. This may be done, for example by mixing the cells with volumes of Dulbecco's Modified Eagle Medium (DMEM) and centrifuging. This provides a pellet of cells that is pure notochord cells. The cells may then be mixed with alginate and the alginate cell solution may be treated so that it forms beads, for example by adding the solution to a solution of calcium chloride. The beads may then be washed and cultured in a medium, for example DMEM, containing one or more infection control substances, for example antibiotics and fungicides, and growth factors, such as fetal calf serum (FCS). The notochord cells (in the form of beads) may be allowed to recover for a period of time before removing the growth factors, for example by using a sodium chloride solution, before culturing in a medium, for example DMEM. This final medium is an example of a notochord enriched medium according to the invention.

Therefore, in more specific embodiments of the present invention, there is provided a method of preparing notochord enriched media comprising:

(a) separating a nucleus pulposus from an intervertabral disc of a nonchondrodystrophic animal to provide a total nucleus digest;

(b) separating notochord cells from the total nucleus digest;

(c) mixing notochord cells with alginate;

(d) converting the alginate-containing notochord cells to beads;

(e) culturing the beads on a medium comprising one or more infection control substances and growth factors;

(f) washing the beads to remove the growth factors; and

(g) reculturing beads in media to provide notochord enriched media.

(iii) Therapeutic Methods of the Invention

The inventors have partially characterized the soluble anabolic factors found in canine notochord enriched media and have found that these factors, in the form of a composition comprising notochord enriched media, in a dose dependent relationship, up-regulate the production of disc chondrocyte proteoglycans, specifically the large, presumably aggregating species. The present inventors have further shown that several genes important to chondrocyte metabolism are increased in expression in both human and bovine disc chondrocytes that have been cultured with notochord enriched media.

Accordingly, the present invention involves a method for enhancing proteoglycan production comprising administering an effective amount of a composition comprising notochord enriched media and/or one or more factors derived therefrom, to a cell or animal in need thereof. The invention also relates to a use of a composition comprising notochord enriched media and/or one or more factors derived therefrom to enhance the production of proteoglycan in a cell or animal in need thereof, and a use of a composition comprising notochord enriched media and/or one or more factors derived therefrom to prepare a medicament to enhance the production of proteoglycan in a cell or animal in need thereof. Preferably the cell is a chondrocyte and the subject is a mammal, in particular, humans.

The term an "effective amount" or a "sufficient amount " of an agent as used herein is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that enhances the production of proteoglycan, an effective amount of an agent is, for example, an amount sufficient to achieve

such an enhancement in proteoglycan production as compared to the response obtained without administration of the agent. An effective amount of the notochord enriched media, or factors derived therefrom, may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the notochord enriched media, or factors derived therefrom, to elicit a desired response in the individual. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

Enhanced production of proteoglycan provides a chondrocytic cell with an ability to maintain its matrix and therefore avoid the internal disruption which otherwise proceeds inexorably with age and/or trauma. Accordingly, in an embodiment of the present invention, there is provided a method for treating degenerative disease of the chondrocyte matrix comprising administering an effective amount of composition comprising notochord enriched media, and/or one or more factors derived from notochord enriched media, to a cell or animal in need thereof. The invention also relates to the use of composition comprising notochord enriched media, and/or one or more factors derived from notochord enriched media, to treat degenerative disease of the chondrocyte matrix in a cell or animal in need thereof, as well as the use of composition comprising notochord enriched media, and/or one or more factors derived from notochord enriched media, to prepare a medicament to treat degenerative disease of the chondrocyte matrix in a cell or animal in need thereof. The chondrocytic cell may be any such cell, including, but not limited to intervertebral chondrocytes and chondrocytes from the articulate cartilage.

In another embodiment of the present invention, the cell in need of treatment is an intervertebral chondrocyte, therefore there is provided a method for treating degenerative disc disease comprising administering to a cell or animal in need of such treatment, an effective amount of a composition comprising notochord enriched media and/or one or more factors derived